# THE RELATIONSHIP OF TYPES OF PHOSPHORUS IN WHEAT FLOURS TO FLOUR QUALITY

by

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# INTRODUCTION AND REVIEW OF LITERATURE

Wheat variety as well as the environmental conditions under which wheat is produced influence the chemical composition of the wheat kernel and the baking quality of the resulting flour. Environment generally exerts the greater influence.

Kent-Jones and Amos (18) and Bailey (3) have summarized the literature on the mineral composition of wheat and flour. Phosphorus is one of the elements present in high concentration in the ash of both wheat and flour. Schrenk (27) studied the effects of calcium, magnesium, and phosphorus fertilizers on the elemental composition of grain produced in Southeastern Kansas. The most noticeable effect of fertilizer application was the increase in phosphorus content of the grain. Bequette, et al. (7) reported that the phosphorus content of gluten obtained from 40 samples of wheat grown in Kansas in 1955-56 was correlated significantly with flour quality. For these samples, many of which were of abnormal quality, phosphorus was more reliable in predicting the breadmaking properties of the flour than protein percentage.

The phosphorus of both wheat and flour may be present as several types of phosphorus compounds. The different types of phosphorus compounds concerned in this report are phytin, phosphatide, ester, nucleic acid, and inorganic.

Phytic acid is an ester derivative of inositol, hexahydroxycyclohexane, and phosphoric acid. Phosphoric acid replaces a hydrogen on the hydroxyl groups of the inositol molecule by splitting off water. Phytin is a calcium-magnesium salt complex of phytic acid.

As reported by Pons, et al. (25), a considerable portion of the phosphorus in the wheat may be in the phytin form. The total quantity of phytin present in wheat varies with the variety, but the majority is located in the bran and

germ and little occurs in the endosperm. Phytic acid phosphorus ranges between 25 to 50 milligrams per 100 grams of flour (18) depending upon the percent of flour extraction. Since the bran and germ contain a high percentage of the total phytic acid, the higher extraction flours also contain more phytic acid phosphorus.

Hlynka (17) showed that phytic acid had no deleterious effect on baking quality when added to dough. It reduced merely the optimum bromate level. Hlynka (17) suggested that the role of complexing agents such as phytic acid might be indirect, causing a decrease in the availability of inorganic ions. This produces favorable conditions for an accelerated reaction of bromate.

Phosphorus also is known to exist in flour in the form of phospholipid compounds which may be either lecithins, cephalins, or phosphatidyl inositol. Phosphatidic acids also occur in plant tissue (15). All phospholipids contain nitrogen and phosphorus, besides carbon, hydrogen, and oxygen. In lecithins and cephalins, the nitrogen-phosphorus ratio is 1:1. The phosphatidic acids are considered to be those compounds in which the base of lecithin or cephalin has been replaced by a metallic ion.

Gortner and Gortner (15) reported that hydrolysis of lecithins yields one molecule of glycerolphosphoric acid, two of fatty acids, and one of choline. Choline is attached to the glycerolphosphoric acid by esterfication of the hydroxyl group of the oxyethyl radical with phosphoric acid. The fatty acids are esterfied on the two remaining hydroxyl groups of glycerol. Stearic, palmitic, oleic, linoleic, and arachidonic acids are present in various lecithins. Since there are two molecules of fatty acid in lecithin, it is evident that a considerable number of isomeric "lecithins" are possible. Thus it is apparent that the term "lecithin" does not mean a specific chemical compound, but rather that this is the generic name of a group of compounds possessing

similarity in structure but likewise possessing specific differences depending on the fatty acids present in the molecule.

The cephalins are similar to the lecithins. Cephalins are usually considered to contain the base ethanolamine or serine instead of choline. However, Gortner and Gortner (15) reported that the crude cephalin obtained from the lipids of many tissues, both plant and animal in origin, contains still another group of phospholipids of which inositol is a constituent.

The role of phospholipids in baking has been of interest to many investigators. Osborne (24) in 1907 reported the presence of a phosphatide, which he called lecithin, in the gluten washed from wheat flour. This was confirmed later by Working (30) and it was suggested that phospholipids affected gluten quality. There was more phosphatide present in the gluten from the lower grades of flour than in the gluten from the higher grades of flour. Furthermore, prolonged washing of the soft gluten from low-grade flour removed phosphatide and gradually increased the tenacity until the gluten practically was equal that from patent flour.

The literature on the phospholipids of wheat and flour has been summarized by Bailey (3) and more recently by Kent-Jones and Amos (18). In general, it was found that the higher the phospholipid content of a flour, the lower its grade, gluten quality, and baking value. There appeared to be an optimum concentration of phosphatide, as reported by Geddes and Larmour (14) and Working (30). Too much phosphatide gave a soft gluten and too little phosphatide a tough and inelastic gluten. Working (30) found that the addition of phosphatides to flour injured the baking quality, whereas Rich (26) and Baker and Mize (4) found that the addition of phosphatides to flour improved baking quality.

Cookson and Coppock (8), in their review of literature on the phosphatides,

stated that much fundamental work has been carried out on the phosphatides of the wheat germ, as well as the acetone-soluble residues of the lipids of other wheat fractions and flour. Phosphatidic acid (as calcium, magnesium, and potassium salts), phosphatidyl choline, and phosphatidyl ethanolamine were identified in these lipid extracts. Faure and Morelac-Coulon (9) reported the isolation of phosphatidyl inositol from wheat. Fisher, et al. (12) suggested that there might be two types of inositol phospholipids in wheat flour. Barton-Wright (5) found the lecithin-cephalin fraction was confined mainly to the envelope of the grain. The remainder of the grain contained principally the magnesium salt of phosphatidic acid.

Gluten prepared by Fisher and Halton (13) contained about three-fourths of the total flour lipids. Sullivan and Near (28) observed the lipid content to be nearly the same in widely different wheats, and found that the ratio of lipid to protein was much greater in soft than in hard wheat.

Tucker (29) observed that removal of the lipids from soft wheat flour increased the amount of coaguable protein that could be recovered on treatment with a sulfite solution. However, the removal of lipids from hard wheat flour did not alter the yield of protein. The addition of flour lipids to previously extracted flour resulted in a smaller yield of protein, regardless of the type of flour from which the lipids were prepared. However, the amount of lipid material required to decrease the protein yield from hard wheat flour was two to three times that required to produce a commensurate decrease in the yield of protein from soft wheat flour. In considering the yield of coagulated protein, it was concluded that the amount of lipid present in the flour was a significant factor, but that the critical amount varied with the type of flour.

Olcott and Mecham (23) found that much of the ether-extractable lipid material of flour was "bound" in doughs. At least three times the amount of

lipid normally present could be bound by the doughing procedure employed. The phospholipids were bound preferentially to other lipids. Most of the "bound" lipid was associated with the gluten, rather than with the nonprotein constituents of the flour. A later report by Mecham and Weinstein (20) showed that sodium chloride as well as a polyoxyethylene stearate type softener decreased the lipid binding of doughs. Lard decreased phospholipid binding slightly but did not affect appreciably the total amount of bound lipid.

Cookson and Coppock (8) stressed the importance of flour lipids in bread-making and noted the presence of a flour lipid, perhaps lipo-protein, which appeared to be an important factor in the mechanism of bread improvement. A later report by Fisher, et al. (12) confirmed the existence of a lipid-protein complex in flour.

Methods of lipid extraction and the efficiency of these methods have been summarized by Mecham and Ali Mohammad (19). No procedure has been devised that will extract all of the lipids from the flour without damaging the protein constituents of the flour either by degradation or denaturation. By using the methods of extraction now available, the flour retains the lipid material most intimately combined with the protein. If better methods were available for extracting lipids from flour, the effects of flour lipids on protein properties and baking behaviour could be determined by comparison with lipid-free material, rather than with material only partially freed of lipids.

The ester type phosphorus compounds are considered to be, for the purpose of this study, the carbohydrate esters of phosphoric acid. The inorganic phosphorus is considered to be the phosphorus that exists in the inorganic state.

The nucleic acids are divided into two general groups, ribonucleic acid and desoxyribonucleic acid. Harrow and Mazur (16) reported that all nucleic acids so far examined tend to resemble one group or the other. Ribonucleic

acid yields phosphoric acid, D-ribose, adenine, guanine, cytosine, and uracil on hydrolysis, while desoxyribonucleic acid yields phosphoric acid, D-2-desoxyribose, adenine, guanine, cytosine, and thymine. It is evident that these two compounds differ only in the sugar component and in the nature of one pyrimidine.

There is virtually little known about the role that ester, inorganic, and nucleic acid types of phosphorus play in wheat and flour quality. Andrews and Bailey (2) reported that a large proportion of the total phosphorus in flour existed in the nucleic acid form, but little as inorganic phosphorus. Pons, et al. (25) found little ester type phosphorus in wheat.

The influence of environment and wheat variety on flour quality is understood only partially. It seems logical to assume, however, that there is a correlation between some of the mineral components of flour and baking quality, especially those elements present in high concentration. It was the objective of this research to study the relationship of the type and concentration of phosphorus compounds present in flour and gluten derived therefrom, to the quality of the flour for breadmaking purposes.

## MATERIALS AND METHODS

The flour samples employed in this study consisted of 40 samples representing five pure varieties of hard red winter wheat grown at eight locations in Kansas in 1956. All wheat samples were milled to a straight-grade flour on an Allis mill (1). Data pertaining to these flour samples are presented in Table 1.

Moisture, ash, and protein were determined according to the methods of the A.A.C.C. (1). The straight-dough baking procedure used to bake the flour samples was the same as that employed by Miller, et al. (21).

Table 1. Protein, ash, and adjusted loaf volume data for the flour samples.

| Station | Variety: Bison | Pawnee | Ponca | Concho | RedChief | Average |
|---|---|---|---|---|---|---|
| | Adjusted Loaf Volume (c.c.)[1] | | | | | |
| Manhattan | 603 | 685 | 680 | 696 | 630 | 659 |
| Hays | 641 | 704 | 657 | 637 | 556 | 639 |
| Colby | 701 | 770 | 736 | 774 | 595 | 715 |
| Garden City | 626 | 627 | 582 | 654 | 633 | 624 |
| Mound Valley | 899 | 804 | 837 | 815 | 769 | 825 |
| Thayer | 822 | 798 | 752 | 756 | 692 | 764 |
| Belleville | 762 | 822 | 836 | 861 | 774 | 811 |
| Canton | 734 | 603 | 708 | 652 | 665 | 672 |
| Average | 724 | 727 | 724 | 731 | 664 | 714 |
| | Flour Protein (percent)[2] | | | | | |
| Manhattan | 17.3 | 17.4 | 17.3 | 16.5 | 17.1 | 17.1 |
| Hays | 17.2 | 15.5 | 16.3 | 15.6 | 15.3 | 16.0 |
| Colby | 14.8 | 14.4 | 14.3 | 13.7 | 13.8 | 14.2 |
| Garden City | 15.2 | 14.9 | 14.9 | 14.7 | 14.1 | 14.8 |
| Mound Valley | 11.3 | 12.1 | 10.7 | 11.0 | 10.6 | 11.1 |
| Thayer | 12.7 | 13.1 | 12.7 | 12.5 | 13.1 | 12.8 |
| Belleville | 13.1 | 12.9 | 12.9 | 12.2 | 13.1 | 12.8 |
| Canton | 15.7 | 17.0 | 15.5 | 16.1 | 15.2 | 15.9 |
| Average | 14.7 | 14.7 | 14.3 | 14.0 | 14.0 | 14.3 |
| | Flour Ash (percent)[2] | | | | | |
| Manhattan | 0.41 | 0.42 | 0.43 | 0.41 | 0.44 | 0.42 |
| Hays | 0.48 | 0.43 | 0.49 | 0.43 | 0.45 | 0.46 |
| Colby | 0.44 | 0.43 | 0.47 | 0.44 | 0.44 | 0.44 |
| Garden City | 0.42 | 0.42 | 0.47 | 0.41 | 0.44 | 0.43 |
| Mound Valley | 0.39 | 0.39 | 0.43 | 0.39 | 0.42 | 0.40 |
| Thayer | 0.40 | 0.42 | 0.41 | 0.37 | 0.37 | 0.39 |
| Belleville | 0.39 | 0.39 | 0.39 | 0.36 | 0.38 | 0.38 |
| Canton | 0.44 | 0.46 | 0.48 | 0.44 | 0.48 | 0.46 |
| Average | 0.42 | 0.42 | 0.45 | 0.41 | 0.43 | 0.42 |

1. (Loaf volume - 200) x 14 / percent flour protein.
2. 14 percent moisture basis.

Adjusted loaf volume, expressed on a 14 percent protein basis, was calculated by subtracting 200 c.c. from the measured loaf volume, dividing by the percent protein of the flour sample and multipling by 14.

The gluten fraction of the flour was the same fraction as that employed by Bequette (6). The procedure of separation was a modification of the method developed by Finney (10). A cold water-flour slurry was made and centrifuged. The dough was removed and kneaded in the supernatant until a separation of the gluten and starch was obtained. The gluten samples were washed several times with cold water and then placed in the refrigerator for one hour after which any remaining liquid was discarded. The gluten samples were then lyophilized at -20° C. The procedure has been described in detail by Bequette (6).

Phosphorus determinations were made colorimetrically according to the methods described by Pons, et al. (25). The final evaluations of total, phytin, and acid-soluble phosphorus involved the use of a reduced molybdate colorimetric method. Acid-soluble phosphorus includes phytin, inorganic, and ester type phosphorus. Inorganic and phosphatide phosphorus determinations utilize a colorimetric method involving extraction of a molybdenum blue complex with isobutyl alcohol. Nucleic acid and ester type phosphorus were obtained by differences of other types of phosphorus. Reference is made to the appendix for details on the procedure for the different types of phosphorus determinations.

## RESULTS AND DISCUSSION

### Baking Quality of the Flours

Many of the flour samples used in this study contained protein of abnormal baking quality (6). Bequette (6) pointed out that an outstanding example of this was the comparison between protein content and loaf volumes of the samples

grown at Hays and Belleville. The Hays samples averaged 16 percent protein and produced bread with an average loaf volume of 930 c.c. The Belleville samples averaged 12.8 percent protein and produced loaves averaging 943 c.c. The samples grown at Manhattan, Garden City, and Canton were considered to contain protein of inferior baking quality, whereas the samples from Colby, Mound Valley, Thayer, and Belleville were of much higher baking quality. The normally expected differences in protein quality among varieties was altered considerably. This alteration was attributed to the effects of environment during the growing season.

Finney and Fryer (11) showed that subnormal loaf volume, water absorption, and mixing properties were associated consistently with high temperatures (above 90° F.) during the last 15 days of ripening. High temperature did not always impair protein quality, however, because of mitigating physical conditions in the soil or relatively high humidity. Varieties with long mixing times were found to be more tolerant to the detrimental effects of high temperature during fruiting than were varieties with short mixing requirements. The summer of 1956 was extremely hot and dry, and it was assumed that these conditions caused the observed abnormal differences in flour quality.

It is commonly accepted that the protein content of wheat flours is the principal factor determining their bread-baking properties. Miller and Johnson (22) reported correlations as high as 0.98 between flour protein and loaf volume. However, for these samples the correlation between loaf volume and protein content ($r = 0.54$) was relatively low (6). This low figure, although significant, provided further evidence that the protein quality of at least some of the samples used in this study were inferior.

Finney (10) showed that the relation between protein content and loaf volume substantially was linear for each variety between the limits of eight

and 20 percent protein for samples grown under normal conditions, and provided an adequate baking formula was used. Therefore, by taking 200 c.c. as the starting point at which loaf volume begins to vary linearly with protein percent, an "adjusted loaf volume" could be calculated as previously described. This calculated "adjusted loaf volume" should eliminate the effects of variable protein and was used in this study for all statistical analyses involving loaf volume.

Protein and adjusted loaf volume data in Table 2 indicate that this method ranked the samples in the proper order of flour quality based on a consideration of flour protein and adjusted loaf volume. This ranking in Table 2 places the varieties and stations in the same order as to the samples with the superior quality as did the gluten quality score method employed by Bequette (6). However, it is believed that the adjusted loaf volume method gives a more accurate single numerical evaluation of the flour quality for these samples than does the gluten quality score.

## Phosphorus Distribution in the Flour and in the Gluten

The distribution of types of phosphorus in the flour and gluten is presented in Tables 3 and 4. A summary of these data by variety and station means is given in Tables 5 and 6. Acid-soluble phosphorus contains the phytin, inorganic, and ester type phosphorus.

For the 40 samples analyzed in this study, acid-soluble phosphorus represented 38.3 percent of the total phosphorus in the flour and 32.4 percent of the total phosphorus in the gluten. However, the amount of phytin, inorganic, and ester type phosphorus in the flour and gluten were found to differ considerably. Inorganic phosphorus represented only 3.0 percent of the total phosphorus of the flour but 14.5 percent of the total phosphorus of the gluten,

Table 2. Summary of variety and station averages for adjusted loaf volume, flour protein, and flour ash for the flour samples.

| Variety or Station | Adjusted Loaf Volume[1] | Flour Protein[2] | Flour Ash[2] |
|---|---|---|---|
| | cc | % | % |
| | | Variety Means | |
| Concho | 731 | 14.0 | 0.41 |
| Pawnee | 727 | 14.7 | 0.42 |
| Ponca | 724 | 14.3 | 0.45 |
| Bison | 724 | 14.7 | 0.42 |
| RedChief | 664 | 14.0 | 0.43 |
| | | Station Means | |
| Mound Valley | 825 | 11.1 | 0.40 |
| Belleville | 811 | 12.8 | 0.38 |
| Thayer | 764 | 12.8 | 0.39 |
| Colby | 715 | 14.2 | 0.44 |
| Canton | 672 | 15.9 | 0.46 |
| Manhattan | 659 | 17.1 | 0.42 |
| Hays | 639 | 16.0 | 0.46 |
| Garden City | 624 | 14.8 | 0.43 |
| Grand Average | 714 | 14.3 | 0.42 |

1. (Loaf Volume - 200) x 14 / percent flour protein.
2. 14 percent moisture basis.

Table 3. Summary of concentration of types of phosphorus in the flour. Expressed as mg. per g. of flour on a 14 percent moisture basis.

| Station & Variety | Total | Acid-Soluble | Inorganic | Phosphatide | Phytin | Nucleic Acid | Ester |
|---|---|---|---|---|---|---|---|
| Manhattan | | | | | | | |
| Bison | 1.170 | 0.323 | 0.042 | 0.035 | 0.012 | 0.812 | 0.269 |
| Pawnee | 1.093 | 0.340 | 0.040 | 0.045 | 0.010 | 0.709 | 0.290 |
| Ponca | 1.097 | 0.335 | 0.035 | 0.038 | 0.019 | 0.724 | 0.281 |
| Concho | 1.007 | 0.274 | 0.028 | 0.044 | 0.000 | 0.689 | 0.246 |
| RedChief | 1.115 | 0.432 | 0.029 | 0.029 | 0.121 | 0.654 | 0.282 |
| Hays | | | | | | | |
| Bison | 1.323 | 0.486 | 0.038 | 0.047 | 0.212 | 0.790 | 0.236 |
| Pawnee | 1.206 | 0.426 | 0.046 | 0.048 | 0.078 | 0.732 | 0.302 |
| Ponca | 1.302 | 0.537 | 0.050 | 0.045 | 0.188 | 0.720 | 0.299 |
| Concho | 1.176 | 0.412 | 0.020 | 0.067 | 0.005 | 0.697 | 0.387 |
| RedChief | 1.301 | 0.594 | 0.019 | 0.040 | 0.146 | 0.667 | 0.429 |
| Colby | | | | | | | |
| Bison | 1.226 | 0.428 | 0.025 | 0.050 | 0.000 | 0.749 | 0.403 |
| Pawnee | 1.271 | 0.439 | 0.024 | 0.055 | 0.068 | 0.777 | 0.347 |
| Ponca | 1.270 | 0.468 | 0.025 | 0.045 | 0.072 | 0.757 | 0.371 |
| Concho | 1.206 | 0.406 | 0.017 | 0.055 | 0.029 | 0.745 | 0.360 |
| RedChief | 1.188 | 0.403 | 0.031 | 0.046 | 0.048 | 0.739 | 0.324 |
| Garden City | | | | | | | |
| Bison | 1.131 | 0.378 | 0.054 | 0.046 | 0.029 | 0.709 | 0.295 |
| Pawnee | 1.144 | 0.540 | 0.057 | 0.049 | 0.061 | 0.555 | 0.422 |
| Ponca | 1.313 | 0.555 | 0.046 | 0.055 | 0.063 | 0.703 | 0.446 |
| Concho | 1.136 | 0.355 | 0.058 | 0.049 | 0.039 | 0.732 | 0.258 |
| RedChief | 1.205 | 0.578 | 0.047 | 0.043 | 0.044 | 0.584 | 0.487 |
| Mound Valley | | | | | | | |
| Bison | 1.066 | 0.361 | 0.031 | 0.067 | 0.019 | 0.638 | 0.311 |
| Pawnee | 1.043 | 0.394 | 0.033 | 0.047 | 0.048 | 0.602 | 0.313 |
| Ponca | 1.047 | 0.400 | 0.032 | 0.046 | 0.019 | 0.601 | 0.349 |
| Concho | 0.962 | 0.341 | 0.035 | 0.061 | 0.027 | 0.560 | 0.279 |
| RedChief | 1.073 | 0.424 | 0.029 | 0.042 | 0.000 | 0.607 | 0.395 |
| Thayer | | | | | | | |
| Bison | 1.105 | 0.415 | 0.043 | 0.053 | 0.094 | 0.637 | 0.278 |
| Pawnee | 1.128 | 0.450 | 0.039 | 0.063 | 0.029 | 0.615 | 0.382 |
| Ponca | 1.109 | 0.397 | 0.030 | 0.048 | 0.073 | 0.664 | 0.294 |
| Concho | 0.944 | 0.414 | 0.032 | 0.057 | 0.010 | 0.473 | 0.372 |
| RedChief | 1.176 | 0.435 | 0.042 | 0.037 | 0.020 | 0.704 | 0.373 |
| Belleville | | | | | | | |
| Bison | 1.077 | 0.377 | 0.032 | 0.041 | 0.056 | 0.659 | 0.289 |
| Pawnee | 1.015 | 0.396 | 0.030 | 0.029 | 0.027 | 0.590 | 0.339 |
| Ponca | 0.953 | 0.481 | 0.026 | 0.051 | 0.097 | 0.421 | 0.358 |
| Concho | 0.896 | 0.397 | 0.019 | 0.059 | 0.003 | 0.440 | 0.375 |
| RedChief | 1.053 | 0.428 | 0.023 | 0.037 | 0.063 | 0.588 | 0.342 |
| Canton | | | | | | | |
| Bison | 1.193 | 0.556 | 0.038 | 0.054 | 0.044 | 0.583 | 0.474 |
| Pawnee | 1.328 | 0.599 | 0.034 | 0.079 | 0.085 | 0.650 | 0.480 |
| Ponca | 1.327 | 0.601 | 0.029 | 0.053 | 0.094 | 0.673 | 0.478 |
| Concho | 1.163 | 0.451 | 0.028 | 0.067 | 0.032 | 0.645 | 0.391 |
| RedChief | 1.344 | 0.541 | 0.030 | 0.035 | 0.159 | 0.769 | 0.352 |
| Average | 1.147 | 0.439 | 0.034 | 0.046 | 0.056 | 0.659 | 0.349 |

Table 4. Summary of concentration of types of phosphorus in the gluten. Expressed as mg. per g. of gluten on a 14 percent moisture basis.

| Station & Variety | Total | Acid-Soluble | Inorganic | Phosphatide | Nucleic Acid | Ester |
|---|---|---|---|---|---|---|
| Manhattan | | | | | | |
| Bison | 1.552 | 0.561 | 0.237 | 0.105 | 0.886 | 0.324 |
| Pawnee | 1.384 | 0.347 | 0.219 | 0.138 | 0.899 | 0.128 |
| Ponca | 1.288 | 0.384 | 0.198 | 0.093 | 0.811 | 0.186 |
| Concho | 1.410 | 0.403 | 0.155 | 0.111 | 0.896 | 0.248 |
| RedChief | 1.642 | 0.586 | 0.298 | 0.079 | 0.977 | 0.288 |
| Hays | | | | | | |
| Bison | 1.763 | 0.663 | 0.216 | 0.121 | 0.979 | 0.447 |
| Pawnee | 1.752 | 0.468 | 0.268 | 0.130 | 1.154 | 0.200 |
| Ponca | 2.016 | 0.855 | 0.307 | 0.112 | 1.049 | 0.548 |
| Concho | 1.741 | 0.517 | 0.238 | 0.123 | 1.101 | 0.279 |
| RedChief | 2.118 | 0.984 | 0.363 | 0.068 | 1.066 | 0.621 |
| Colby | | | | | | |
| Bison | 1.820 | 0.554 | 0.211 | 0.117 | 1.149 | 0.343 |
| Pawnee | 1.688 | 0.579 | 0.255 | 0.089 | 1.020 | 0.324 |
| Ponca | 1.782 | 0.570 | 0.256 | 0.115 | 1.097 | 0.314 |
| Concho | 1.648 | 0.517 | 0.227 | 0.160 | 0.971 | 0.290 |
| RedChief | 1.794 | 0.748 | 0.288 | 0.082 | 0.964 | 0.460 |
| Garden City | | | | | | |
| Bison | 1.820 | 0.692 | 0.332 | 0.157 | 0.971 | 0.360 |
| Pawnee | 1.734 | 0.621 | 0.293 | 0.098 | 1.015 | 0.328 |
| Ponca | 1.910 | 0.741 | 0.289 | 0.099 | 1.070 | 0.452 |
| Concho | 1.738 | 0.696 | 0.232 | 0.089 | 0.953 | 0.464 |
| RedChief | 1.893 | 0.668 | 0.316 | 0.081 | 1.144 | 0.352 |
| Mound Valley | | | | | | |
| Bison | 1.540 | 0.345 | 0.146 | 0.186 | 1.009 | 0.199 |
| Pawnee | 1.643 | 0.347 | 0.194 | 0.154 | 1.142 | 0.153 |
| Ponca | 1.563 | 0.355 | 0.217 | 0.195 | 1.013 | 0.138 |
| Concho | 1.334 | 0.336 | 0.149 | 0.148 | 0.850 | 0.187 |
| RedChief | 1.546 | 0.391 | 0.233 | 0.143 | 1.012 | 0.158 |
| Thayer | | | | | | |
| Bison | 1.536 | 0.406 | 0.219 | 0.211 | 0.919 | 0.187 |
| Pawnee | 1.444 | 0.306 | 0.130 | 0.159 | 0.979 | 0.176 |
| Ponca | 1.525 | 0.431 | 0.194 | 0.104 | 0.990 | 0.237 |
| Concho | 1.486 | 0.238 | 0.166 | 0.154 | 1.094 | 0.072 |
| RedChief | 1.470 | 0.506 | 0.244 | 0.150 | 0.814 | 0.262 |
| Belleville | | | | | | |
| Bison | 1.381 | 0.332 | 0.164 | 0.189 | 0.860 | 0.168 |
| Pawnee | 1.561 | 0.331 | 0.212 | 0.107 | 1.123 | 0.119 |
| Ponca | 1.442 | 0.337 | 0.171 | 0.148 | 0.957 | 0.166 |
| Concho | 1.345 | 0.263 | 0.124 | 0.143 | 0.939 | 0.139 |
| RedChief | 1.516 | 0.417 | 0.212 | 0.124 | 0.975 | 0.205 |
| Canton | | | | | | |
| Bison | 1.768 | 0.738 | 0.295 | 0.094 | 0.936 | 0.443 |
| Pawnee | 1.700 | 0.730 | 0.323 | 0.161 | 0.809 | 0.407 |
| Ponca | 1.838 | 0.730 | 0.292 | 0.128 | 0.980 | 0.438 |
| Concho | 1.553 | 0.554 | 0.245 | 0.191 | 0.808 | 0.309 |
| RedChief | 2.107 | 1.046 | 0.397 | 0.116 | 0.945 | 0.649 |
| Average | 1.657 | 0.532 | 0.238 | 0.129 | 0.983 | 0.294 |

Table 5. Summary of variety and station averages for concentration of types of phosphorus in the flour. Expressed as mg. per g. of flour on 14% M.B.

| Variety or Station | Total | Acid-Soluble | Inorganic | Phosphatide | Phytin | Nucleic Acid | Ester |
|---|---|---|---|---|---|---|---|
| | | | | Variety Means | | | |
| Concho | 1.061 | 0.381 | 0.030 | 0.057 | 0.018 | 0.623 | 0.334 |
| Pawnee | 1.154 | 0.448 | 0.038 | 0.052 | 0.051 | 0.654 | 0.359 |
| Ponca | 1.177 | 0.472 | 0.034 | 0.048 | 0.078 | 0.658 | 0.360 |
| Bison | 1.161 | 0.416 | 0.038 | 0.049 | 0.058 | 0.697 | 0.319 |
| RedChief | 1.182 | 0.479 | 0.031 | 0.039 | 0.075 | 0.664 | 0.373 |
| | | | | Station Means | | | |
| Mound Valley | 1.038 | 0.384 | 0.032 | 0.053 | 0.023 | 0.602 | 0.329 |
| Belleville | 0.999 | 0.416 | 0.026 | 0.043 | 0.049 | 0.540 | 0.341 |
| Thayer | 1.092 | 0.422 | 0.037 | 0.052 | 0.045 | 0.619 | 0.340 |
| Colby | 1.232 | 0.429 | 0.024 | 0.050 | 0.043 | 0.753 | 0.361 |
| Canton | 1.271 | 0.550 | 0.032 | 0.058 | 0.083 | 0.664 | 0.435 |
| Manhattan | 1.096 | 0.341 | 0.035 | 0.038 | 0.032 | 0.718 | 0.274 |
| Hays | 1.262 | 0.491 | 0.035 | 0.049 | 0.126 | 0.721 | 0.331 |
| Garden City | 1.186 | 0.481 | 0.052 | 0.048 | 0.047 | 0.657 | 0.382 |
| Grand Average | 1.147 | 0.439 | 0.034 | 0.049 | 0.056 | 0.659 | 0.349 |
| Percent of Total[1] | 100 | 38.3 | 3.0 | 4.3 | 4.9 | 57.4 | 30.4 |

1. Average of the 40 samples.

Table 6. Summary of variety and station averages for concentration of types of phosphorus in the gluten. Expressed as mg. per g. of gluten on 14% M.B.

| Variety or Station | Total | Acid-Soluble | Inorganic | Phosphatide | Nucleic Acid | Ester |
|---|---|---|---|---|---|---|
| | | | Variety Means | | | |
| Concho | 1.532 | 0.440 | 0.192 | 0.140 | 0.952 | 0.248 |
| Pawnee | 1.613 | 0.466 | 0.237 | 0.130 | 1.018 | 0.229 |
| Ponca | 1.671 | 0.550 | 0.240 | 0.124 | 0.996 | 0.310 |
| Bison | 1.648 | 0.536 | 0.228 | 0.148 | 0.964 | 0.309 |
| RedChief | 1.761 | 0.668 | 0.294 | 0.105 | 0.987 | 0.374 |
| | | | Station Means | | | |
| Mound Valley | 1.525 | 0.355 | 0.188 | 0.165 | 1.005 | 0.167 |
| Belleville | 1.449 | 0.336 | 0.177 | 0.142 | 0.971 | 0.159 |
| Thayer | 1.492 | 0.377 | 0.191 | 0.156 | 0.959 | 0.187 |
| Colby | 1.746 | 0.594 | 0.247 | 0.113 | 1.040 | 0.346 |
| Canton | 1.793 | 0.760 | 0.310 | 0.138 | 0.896 | 0.449 |
| Manhattan | 1.455 | 0.456 | 0.221 | 0.105 | 0.894 | 0.235 |
| Hays | 1.878 | 0.697 | 0.278 | 0.111 | 1.070 | 0.419 |
| Garden City | 1.819 | 0.684 | 0.292 | 0.105 | 1.031 | 0.391 |
| Grand Average | 1.645 | 0.532 | 0.238 | 0.129 | 0.983 | 0.294 |
| Percent of Total[1] | 100 | 32.4 | 14.5 | 7.9 | 59.8 | 17.9 |

1. Average of the 40 samples.

Table 7. Summary of mean squares and F values from analyses of variance for the different types of phosphorus in the flour.

| Variables | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| | Total Phosphorus | | |
| Varieties | 4 | 0.0194 | 7.34*** |
| Stations | 7 | 0.0546 | 20.59*** |
| Error | 28 | 0.0026 | |
| Total | 39 | | |
| | Acid-Soluble Phosphorus | | |
| Varieties | 4 | 0.0134 | 6.44*** |
| Stations | 7 | 0.0216 | 10.38*** |
| Error | 28 | 0.0021 | |
| Total | 39 | | |
| | Nucleic Acid Phosphorus | | |
| Varieties | 4 | 0.0565 | 1.30 ns |
| Stations | 7 | 0.0253 | 5.80*** |
| Error | 28 | 0.0044 | |
| Total | 39 | | |
| | Inorganic Phosphorus | | |
| Varieties | 4 | 0.00012 | 3.50* |
| Stations | 7 | 0.00037 | 10.39*** |
| Error | 28 | 0.000036 | |
| Total | 39 | | |
| | Phosphatide Phosphorus | | |
| Varieties | 4 | 0.00037 | 6.18*** |
| Stations | 7 | 0.00018 | 3.06* |
| Error | 28 | 0.00006 | |
| Total | 39 | | |
| | Ester Phosphorus | | |
| Varieties | 4 | 0.0038 | 1.21 ns |
| Stations | 7 | 0.0108 | 3.43** |
| Error | 28 | 0.0032 | |
| Total | 39 | | |
| | Phytin Phosphorus | | |
| Varieties | 4 | 0.0063 | 4.44** |
| Stations | 7 | 0.0055 | 3.88*** |
| Error | 28 | 0.0014 | |
| Total | 39 | | |

Table 8. Summary of mean squares and F values from analyses of variance for the different types of phosphorus in the gluten.

| Variables | Degrees of Freedom | Mean Square | F |
|---|---|---|---|
| | Total Phosphorus | | |
| Varieties | 4 | 0.0558 | 5.42*** |
| Stations | 7 | 0.1636 | 15.88*** |
| Error | 28 | 0.0103 | |
| Total | 39 | | |
| | Acid-Soluble Phosphorus | | |
| Varieties | 4 | 0.0632 | 8.29*** |
| Stations | 7 | 0.1467 | 19.25*** |
| Error | 28 | 0.0076 | |
| Total | 39 | | |
| | Nucleic Acid Phosphorus | | |
| Varieties | 4 | 0.0055 | 0.74 ns |
| Stations | 7 | 0.0212 | 2.85* |
| Error | 28 | 0.0074 | |
| Total | 39 | | |
| | Inorganic Phosphorus | | |
| Varieties | 4 | 0.0035 | 1.88 ns |
| Stations | 7 | 0.0134 | 7.22*** |
| Error | 28 | 0.0018 | |
| Total | 39 | | |
| | Phosphatide Phosphorus | | |
| Varieties | 4 | 0.0021 | 2.80* |
| Stations | 7 | 0.0029 | 3.85*** |
| Error | 28 | 0.0008 | |
| Total | 39 | | |
| | Ester Phosphorus | | |
| Varieties | 4 | 0.0264 | 4.40*** |
| Stations | 7 | 0.0722 | 12.05*** |
| Error | 28 | 0.0060 | |
| Total | 39 | | |

or 7.8 and 44.8 percent of the acid soluble phosphorus in the flour and gluten respectively. This low percent of inorganic phosphorus found in the flour agreed with the work of Andrews and Bailey (2).

Phytin phosphorus existed in small amounts in the flour fraction, ranging from an undetectable amount in some samples to as high as 11.8 percent of the phosphorus in one sample. The average phytin phosphorus concentration of these flour samples was 4.9 percent of the total phosphorus. The wide range of the amount of phytin phosphorus would indicate that some of the flour samples had larger amounts of bran. Phytin phosphorus is known to be more concentrated in the bran and germ of the wheat kernel than in the endosperm. An analysis of mean values for phytin phosphorus and percent flour ash showed that in each case the varieties of higher ash content also contained more phytin phosphorus than the varieties of lower ash content. This agreed reasonably well with the literature (18). The amount of phytin phosphorus in the gluten was too small to be detected by the methods employed in this study. However, since very little phytin phosphorus occured in the flour samples in most cases, it can be assumed that there would be virtually no phytin phosphorus in the gluten.

Ester type phosphorus was found to be of reasonably high concentration in both the flour and gluten but was nearly two times as concentrated in the flour when compared to the total phosphorus. This type of phosphorus represented 30.4 percent of the total flour phosphorus and 17.9 percent of the total gluten phosphorus.

Nucleic acid phosphorus consistently was high in both the flour and gluten samples. The percent of the total phosphorus that was of the nucleic acid type in the flour and gluten fraction was 57.4 and 59.8 percent respectively. Andrews and Bailey (2) found that a major portion of the phosphorus in the flour was of the nucleic acid type.

to the differences of this type of phosphorus in the gluten. Location was a highly significant factor accounting for the differences of ester phosphorus in both the flour and gluten.

Variety was a significant and a more important factor than location in affecting the differences of concentration of both phosphatide and phytin phosphorus in the flour. However, location was a significant factor affecting the differences of both phytin and phosphatide phosphorus in the flour. The reverse was true for the affect of variety and location affect on differences of phosphatide phosphorus in the gluten. In this case, location had a greater affect than variety but both variety and location were significant factors.

Variety was an insignificant factor attributing to the differences of nucleic acid phosphorus in both the flour and gluten. Location was a significant factor accounting for the differences of nucleic acid phosphorus in the flour and gluten, but of less significance in the gluten than in the flour.

### Relationship of Types of Phosphorus to Baking Quality

The method of expressing the different elemental components in the gluten is somewhat different than that employed by Bequette (6). The method employed by Bequette (6) expressed the concentration of the elements as the amount in the gluten obtained from 100 g. of flour. This method, under statistical treatment, took into consideration the amount of gluten obtained from the 100 g. of flour in each case and carried with it this variable in all statistical analyses. The method employed in this study was to use the actual concentration (mg. per g.) of the different phosphorus components in the flour and gluten fraction. Thus the procedure employed in this study did not take into consideration the amount of gluten obtained from the flour and was concerned

only with the concentration of the types of phosphorus in this fraction. Therefore, a variable, the amount of gluten was eliminated. This method of expression appeared more logical considering the objective of this study and accordingly the statistical analyses would have greater significance as applied to this research problem.

The correlation coefficients between the concentration of the various types of phosphorus in the flour and gluten and the adjusted loaf volume are presented in Table 9. The simple coefficients of correlation between adjusted loaf volume and types of flour phosphorus indicated that total and nucleic acid phosphorus were the only factors affecting baking quality to any great extent. The phosphorus correlation coefficients between adjusted loaf volume and total and nucleic acid were -0.635 and -0.511 respectively. Acid-soluble and inorganic phosphorus, though significantly correlated with adjusted loaf volume were of too low a magnitude for any practical purpose.

The simple correlation coefficients between all types of phosphorus, with exception of nucleic acid phosphorus, in the gluten and adjusted loaf volume were highly significant. It was noted that the correlations between adjusted loaf volume and the total phosphorus in the flour or in the gluten were of the same magnitude. However, the nucleic acid type phosphorus decreased from a negative significant value for the flour to a positive non-significant correlation for the gluten. Other correlations between adjusted loaf volume and all other types of phosphorus in the gluten increased to a high degree of significance as compared to either a correlation of low order or of insignificant value for the comparable types of flour phosphorus.

Table 9 shows an exceedingly high correlation between acid-soluble, inorganic, ester, and phosphatide phosphorus in the gluten and adjusted loaf

Table 9. Correlation coefficients between types of phosphorus (mg. per g.) in the flour and in the gluten with adjusted loaf volume. D. F. = 38.

| Flour | | Gluten | |
|---|---|---|---|
| Type of Phosphorus | Correlation Coefficient | Type of Phosphorus | Correlation Coefficient |
| Total | -0.635*** | Total | -0.574*** |
| Acid-Soluble | -0.361* | Acid-Soluble | -0.731*** |
| Inorganic | -0.328* | Inorganic | -0.709*** |
| Ester | -0.160 ns | Ester | -0.690*** |
| Phosphatide | 0.158 ns | Phosphatide | 0.558*** |
| Nucleic Acid | -0.511*** | Nucleic Acid | 0.048 ns |
| Phytin | -0.299 ns | | |

ns = non significant
* = significant at the 5 percent level
** = significant at the 1 percent level
*** = significant at the .1 percent level

Table 10. Multiple and partial correlation coefficients between adjusted loaf volume and inorganic, phosphatide, and ester type gluten phosphorus.

| Multiple Correlations | | | Partial Correlations | | |
|---|---|---|---|---|---|
| Variables | D.F. | R | Variables | D.F. | Coeff. |
| $Y; X_1, X_2$ | 37 | 0.761*** | $Y \& X_1; X_2 \& X_3$ | 36 | -0.372* |
| $Y; X_1, X_2, X_3$ | 36 | 0.774*** | $Y \& X_2; X_1 \& X_3$ | 36 | 0.332* |
| | | | $Y \& X_3; X_1 \& X_2$ | 36 | -0.215 ns |

Y = adjusted loaf volume
$X_1$ = inorganic phosphorus
$X_2$ = phosphatide phosphorus
$X_3$ = ester phosphorus
ns = non significant
* = significant at the 5 percent level
*** = significant at the .1 percent level

volume in each case. Multiple and partial correlations were performed in order to determine which of these types of phosphorus was contributing most to the baking quality of the flours. However, since inorganic and ester type phosphorus make up the total amount of acid-soluble phosphorus in the gluten, acid-soluble phosphorus was eliminated from the multiple and partial correlation calculations. The results of these analyses are presented in Table 10.

The multiple correlations showed little increase over the simple correlation between adjusted loaf volume and inorganic phosphorus. The partial correlations showed that adjusted loaf volume was significantly correlated (-0.372) with inorganic phosphorus when phosphatide and ester type phosphorus were held constant. A similar positive partial correlation (0.332) was obtained for the phosphatide phosphorus when the inorganic and ester type phosphorus were held constant. However, this was not the case with the ester type phosphorus. The insignificant partial correlation (-0.215) obtained for ester type phosphorus indicated that this type of phosphorus did not affect baking quality when inorganic and phosphatide phosphorus were held constant. However, it can be argued that there may not be a significant difference between any one of the three partial correlations.

Further study of the correlations of these three types of phosphorus with adjusted loaf volume indicated that both inorganic and phosphatide phosphorus contributed to the increase in the value of the multiple linear regression ($F = 6.79^{*}$ when phosphatide phosphorus was added after inorganic phosphorus). However, little was contributed to the multiple linear regression by the ester type phosphorus after the inorganic and phosphatide phosphorus were added. This was based on the partial correlation (-0.215 ns) or the F value (1.75 ns) obtained when ester type phosphorus was included with the inorganic and phosphatide phosphorus in the multiple linear regression.

Table 11. Correlation coefficients between types of phosphorus (mg. per g.) in the flour and in the gluten with percent flour protein. D.F. = 38.

| Flour | | Gluten | |
|---|---|---|---|
| Type of Phosphorus | Correlation Coefficient | Type of Phosphorus | Correlation Coefficient |
| Total | 0.546*** | Total | 0.314* |
| Acid-Soluble | 0.194 ns | Acid-Soluble | -0.510*** |
| Inorganic | 0.202 ns | Inorganic | 0.457** |
| Ester | -0.463** | Ester | 0.499** |
| Phosphatide | -0.107 ns | Phosphatide | -0.447** |
| Nucleic Acid | 0.542*** | Nucleic Acid | -0.200 ns |
| Phytin | 0.330* | | |

ns = non significant
* = significant at the 5 percent level
** = significant at the 1 percent level
*** = significant at the .1 percent level

The relationships that existed between percent flour protein and types of phosphorus for these samples are presented in Table 11. The correlation coefficients obtained by this method of statistical treatment approach those obtained for similar correlation coefficients with adjusted loaf volume, except that in most cases the correlations are of lower magnitude and of opposite sign. Total and nucleic acid phosphorus in the flour were highly significantly correlated with percent flour protein. The correlation coefficients were 0.546 and 0.542 for total and nucleic acid phosphorus, respectively. Ester type phosphorus in the flour also was correlated significantly (-0.463) with flour protein, whereas with adjusted loaf volume a similar correlation was insignificant. Ester type phosphorus was negatively correlated in both cases and was the only type of phosphorus that retained the same sign in both correlations. Acid-soluble and inorganic phosphorus in the flour both dropped from significant correla-

tions against adjusted loaf volume to insignificant correlations against flour protein. Phosphatide phosphorus remained nearly the same magnitude in both cases but of opposite sign.

Correlation coefficients obtained for types of phosphorus in the gluten fraction are all significant except the nucleic acid type. Nucleic acid type phosphorus in the gluten was not significantly correlated with adjusted loaf volume. In each case, the correlations obtained for percent flour protein and the phosphorus data was lower than the same correlations for adjusted loaf volume. The only type of phosphorus in the gluten that retained the same sign when correlated with adjusted loaf volume and percent flour protein was the acid-soluble phosphorus.

## SUMMARY AND CONCLUSIONS

Forty samples of flour and the gluten fraction of these flours, representing five pure hard red winter wheat varieties grown at different locations in Kansas in 1956 were analyzed for different types of phosphorus. The types of phosphorus included total, acid-soluble, nucleic acid, phytin, inorganic, phosphatide, and ester. The concentration of the different types of phosphorus in the flour and gluten fraction was correlated with adjusted loaf volume and percent flour protein. Adjusted loaf volume was used as a single numerical evaluation of flour quality for these samples, many which were of poor baking quality.

This investigation of the concentration of the different types of phosphorus in the flour and gluten has led to the following conclusions:

The average distribution of the types of phosphorus in the flour, based on the total phosphorus in the flour and expressed as mg. per g. of flour, was acid-soluble 38.3 percent, nucleic acid 57.4 percent, phytin 4.9 percent,

inorganic 3.0 percent, phosphatide 4.3 percent, and ester 30.4 percent. The average distribution of the types of phosphorus in the gluten, based on the total phosphorus in the gluten and expressed as mg. per g. of gluten, was acid-soluble 32.4 percent, nucleic acid 59.8 percent, inorganic 14.5 percent, phosphatide 7.9 percent, and ester 17.9 percent. No phytin phosphorus was found in the gluten fraction.

Location and variety generally both were significant factors in determining the concentration of the different types of phosphorus in the flour and gluten. Location generally was more important than variety in determining the concentration of the different types of phosphorus.

Concentration of total phosphorus in the flour was a better measure of flour quality of these samples than was flour protein. The correlations between adjusted loaf volume and concentration of total phosphorus in the gluten and between loaf volume and flour protein content were of the same magnitude.

The correlation between acid-soluble phosphorus in the flour and adjusted loaf volume was of too low a magnitude for flour quality prediction purposes, but the correlation between this type of phosphorus in the gluten and adjusted loaf volume was a reliable means of estimating flour quality.

Nucleic acid phosphorus concentration in the flour was correlated significantly and negatively with adjusted loaf volume. The correlation between the concentration of nucleic acid phosphorus in the gluten and adjusted loaf volume was insignificant.

Little phytin phosphorus occured in the flour samples and varied with percent flour ash. No phytin phosphorus could be detected in the gluten by the method employed in this study. An insignificant relationship existed between flour quality and concentration of phytin phosphorus in the flour.

The correlation coefficients between the concentration of inorganic,

phosphatide, and ester phosphorus in the flour and adjusted loaf volume indicated in each case that these three types of phosphorus had little affect on flour quality. However, the correlation between the concentration of inorganic, phosphatide, and ester phosphorus in the gluten and adjusted loaf volume were reliable means of predicting the quality of the flour samples 50 percent of the time.

The value of the simple correlation coefficient between adjusted loaf volume and inorganic phosphorus in the gluten was not increased appreciably by a multiple correlation between adjusted loaf volume and inorganic, phosphatide, and ester phosphorus.

Partial correlations involving inorganic, phosphatide, and ester phosphorus indicated that inorganic phosphorus was a reliable means of predicting flour quality when phosphatide and ester phosphorus were held constant. Similarly, phosphatide phosphorus was correlated significantly with adjusted loaf volume when inorganic and ester phosphorus were held constant. However, ester phosphorus was not significantly correlated with adjusted loaf volume when inorganic and phosphatide phosphorus were held constant.

A multiple correlation between adjusted loaf volume and inorganic, phosphatide and ester phosphorus indicated that inorganic and phosphatide phosphorus contributed significantly to the value of a multiple linear regression. However, ester phosphorus contributed little to the value of this multiple linear regression.

The relationship that existed between types of phosphorus and flour protein content generally approached similar relationships that existed between adjusted loaf volume and types of phosphorus, except that in most cases the former coefficients of correlations were of lower magnitude and of opposite sign.

## SUGGESTIONS FOR FUTURE WORK

This investigation was designed to determine which types of phosphorus in flour were related to baking quality. The results of this investigation have suggested the following areas for future research.

An investigation of the concentration of the different types of phosphorus in the plant and wheat kernel during the growing season and relating this data to flour quality.

A study of the concentration of different types of phosphorus in the wheat kernel and flour as related to different levels of phosphorus fertilization.

An investigation to determine how the different types of phosphorus alter the physical and chemical properties of dough.

A study to develop a method of extracting all of the lipids from flour without injury to the protein and study the effects of addition of different concentration of these extracts to the breadmaking properties of the flour.

Identification of the types and concentration of phospholipids in flour and flour fractions and an investigation leading to the role that these phospholipids have in affecting flour quality from a physical and chemical viewpoint.

A study of the relationship among the different types of phosphorus in the wheat kernel, flour, and flour fraction and other elemental components.

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# APPENDIX

Reduced Molybdate Colorimetric Determination of Phosphorus (25)

Preparation of Solutions. The concentrated reduced molybdate reagent was prepared by weighing 39.12 g. of reagent grade molybdic anhydride ($MoO_3$) into a two liter, round-bottom pyrex flask with two necks and adding 800 ml. of concentrated sulfuric acid. A mechanical driven glass stirring rod was introduced through one neck of the flask and a thermometer through the other neck. The solution was heated, with continuous stirring on an electrically heated mantle, at 150° C. until solution was complete (1.5 to 2.0 hrs.) as indicated by a clear, greenish color. After the quantitative addition of 2.20 g. of powdered molybdenum metal (99.5 percent Mo), heating and stirring was resumed until the solution was complete. This required about two hours. The deep blue solution was cooled, transferred quantitatively to a one liter volumetric flask, and diluted to volume with concentrated sulfuric acid.

The dilute reduced molybdate solution was prepared by pipeting 10 ml. of the concentrated reduced molybdate reagent into a 100 ml. volumetric flask containing about 50 ml. of distilled water. Because of the viscosity of the reagent, the inside of the pipete was washed with distilled water into the flask. The dilute reagent was cooled to room temperature and diluted to volume with distilled water. A fresh solution of this reagent was prepared each day.

The concentrated stock phosphate solution was prepared by dissolving 4.3929 g. of A.C.S. grade dry monobasic potassium phosphate in 300 ml. of distilled water and 200 ml. of N sulfuric acid contained in a one liter volumetric flask. Several drops of 0.1 N potassium permanganate were added and solution diluted to volume with distilled water. This solution contained 1.0 mg. of phosphorus per ml. and was stable.

The dilute stock phosphate solution containing 0.01 mg. of phosphorus per

ml. was prepared by diluting the concentrated stock solution one to 100. A fresh solution of this solution was prepared each time before use.

Digestion of Sample. An appropriate amount of sample was weighed, or a suitable aliquot of extract pipeted, into a 100 ml. micro-Kjeldahl flask. Three ml. of concentrated sulfuric acid and two, six mm., glass beads were added. The sample was heated until all organic material was charred and a homogeneous solution obtained. After cooling, four drops of 30 percent hydrogen peroxide were added and the solution was heated until colorless. It usually was necessary to add additional peroxide up to a total of 10 drops with intermittent heating and cooling. The solution was heated for 10 minutes after the last addition of peroxide. When cool, 20 ml. of distilled water was added and the solution boiled for five minutes to remove any remaining peroxide and to insure conversion of phosphorus to the ortho form. After cooling, the solution was quantitatively transferred to a 100 ml. volumetric flask and diluted to volume. This sample was used for the colorimetric determination of phosphorus.

Colorimetric Determination of Phosphorus. A suitable aliquot of the sample solution was transferred to a 100 ml. volumetric flask. Sufficient 3.60 N sodium hydroxide was added to neutralize the acid. Two drops of indicator, 0.2 percent aqueous solution of sodium alizarin sulfonate, were added and the acidity adjusted with 1 N sulfuric acid and 1 N sodium hydroxide until one drop of the acid turned the solution yellow. The solution was then diluted to approximately 70 ml. with distilled water. A reagent blank was prepared using the same amount of 3.60 N alkali as for the samples and the acidity adjusted in the same manner. A ten ml. aliquot of the dilute reduced molybdate reagent was added to the blank and each sample solution. All flasks were mixed by swirling and placed in a boiling water bath for exactly 30 minutes. After cooling in a cold water bath, the reaction solution were diluted to volume

with distilled water. Intensity of the color was read in a Bausch and Lomb "Spectronic 20" colorimeter at 820 mu. Using the reagent blank, the instrument was set at 100 percent transmission. Milligrams of phosphorus in the sample aliquot was determined by reference to the calibration curve.

Calibration Curve. Aliquots of 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, and 12.0 ml. of the diluted phosphate solution (0.01 mg. of phosphorus per ml.) were pipeted into 100 ml. volumetric flask. Two drops of indicator and one drop of 1 N sulfuric acid were added and the solution diluted to approximately 70 ml. with distilled water. After adding 10 ml. of reduced molybdate solution, the procedure outlined above for treatment of the sample aliquot was followed. The standard containing no phosphorus was used to set the instrument at 100 percent transmission. The logarithms of the transmittance values obtained for the standards were plotted against the known phosphorus concentrations to obtain the standard calibration curve.

Isobutyl Alcohol Colorimetric Determination of Phosphorus (25)

Preparation of Solutions. The molybdate reagent was prepared by dissolving 50 g. of ammonium molybdate in 400 ml. of 10 N sulfuric acid. This was diluted to one liter with distilled water.

The stannous chloride stock solution was made by dissolving 10 g. of stannous chloride dihydrate in 25 ml. of concentrated hydrochloric acid. This stock solution was stored in a glass stoppered brown bottle.

The dilute stannous chloride solution was prepared by diluting one ml. of the stock solution to 200 ml. with N sulfuric acid. This solution was prepared just before use because it is not stable.

The stock and dilute phosphorus solutions were prepared as previously described under the reduced molybdate method.

Colorimetric Determination of Phosphorus. A suitable aliquot of an extract or of a digested sample was pipeted into a 125 ml. separatory funnel. Five ml. of the molybdate reagent was added and the solution diluted to 20 ml. with distilled water. Ten ml. of isobutyl alcohol was added and the funnels were shaken for two minutes. At the end of this extraction time the aqueous layer was discarded and the solution washed by shaking once for 0.5 minutes with 10 ml. of N sulfuric acid, again discarding the aqueous layer. Fifteen ml. of the dilute stannous chloride solution was added, and the solution shaken for one minute. The aqueous layer was discarded. The blue isobutyl alcohol layer was quantitatively transferred to a 50 ml. volumetric flask using 95 percent ethyl alcohol to effect the transfer and diluted to volume with 95 percent ethyl alcohol. A sample blank was determined in the same manner as above using all reagents.

Intensity of the color was read in a Bausch and Lomb "Spectronic 20" colorimeter at 720 mu using the reagent blank to set the instrument at 100 percent transmittance. Milligrams of phosphorus in the sample aliquot was determined by reference to the calibration curve.

Calibration Curve. A calibration curve was prepared by pipeting 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. of the dilute phosphate solution (0.01 mg. of phosphorus per ml.) into 125 ml. separatory funnels. The color was developed exactly as outlined above for the sample determination. The standard containing no phosphorus was used to set the instrument at 100 percent transmission. The logarithms of the transmittance values for the standards were treated statistically as outlined in the reduced molybdate method.

Methods for Determining Phosphorus Distribution (25)

Preparation of Solutions. The 0.75 N trichloracetic acid solution was prepared by dissolving 123 g. of reagent grade acid in distilled water and diluting to one liter. This acid solution was either made as needed or stored in a refrigerator.

The benzene-alcohol azeotropic solvent was made by mixing 32.4 weight percent of absolute ethyl alcohol and 67.6 weight percent of benzene. This solvent has a boiling point of 68.2° C.

The magnesium nitrate solution used was a saturated solution of magnesium nitrate hexahydrate in 95 percent ethyl alcohol.

The two percent hydrochloric acid solution containing sodium sulfate was prepared by dissolving 100 g. of anhydrous sodium sulfate in about 500 ml. of distilled water contained in a one liter volumetric flask. After the sodium sulfate was dissolved, 48.5 ml. of concentrated hydrochloric acid was added and the solution made up to volume.

A 0.6 percent hydrochloric acid solution containing sodium sulfate was prepared in the same manner as the two percent solution except 14.5 ml. of concentrated hydrochloric acid was used in place of the 48.5 ml. of concentrated hydrochloric acid.

The ferric chloride reagent was prepared by dissolving 15.0 g. of ferric chloride hexahydrate, $FeCl_3.6H_2O$, in 1 N hydrochloric acid and diluting to one liter with 1 N hydrochloric acid.

Total Phosphorus. Total phosphorus was determined by digesting 400 mg. of flour or 300 mg. of gluten as outlined under the reduced molybdate method. Colorimetric determinations also were made by the reduced molybdate colorimetric procedure.

Acid-Soluble Phosphorus. Acid-soluble phosphorus was determined by weighing one g. of flour or 500 mg. of gluten into a 11.0 cm. filter paper, Whatman No. 42, The filter paper was folded, and enclosed in a second so as to retain the sample. The second filter paper was left open at the top to form a thimble. The sample was extracted with Skelly Solve B in a Soxhlet apparatus for four hours. After the extraction period, the sample was air dried for 24 hours and transferred quantitatively to a 125 ml. glass stoppered erlenmeyer flask. Seventy-five ml. of 0.75 N trichloracetic acid was pipeted into the flask and the sample extracted on a mechanical shaker for one hour. The sample was centrifuged at high speed using 50 ml. polyethylene centrifuge tubes in a small laboratory centrifuge and then filtered through 11 cm. filter paper, Whatman No. 42, discarding the first few drops of the filtrate. A 15 ml. aliquot of this filtrate was digested as outlined under the reduced molybdate method. A 10 ml. aliquot of the digested sample was used for the colorimetric determination of phosphorus by the reduced molybdate procedure.

Inorganic Phosphorus. A five ml. aliquot of the trichloracetic acid filtrate from the acid-soluble phosphorus determination was pipeted into a 125 ml. separatory funnel. Inorganic phosphorus was determined by the isobutyl alcohol procedure.

Phosphatide Phosphorus. A two g. sample of flour or a one g. sample of gluten was weighed into a 11 cm. filter paper, Whatman No. 42. The filter paper was folded, and enclosed in a second filter paper so as to retain the sample. The second filter paper was left open at the top to form a thimble. The sample was extracted with 75 ml. of the alcohol-benzene solvent for two hours in a small size Soxhlet extractor. The sample was removed, air dried for two hours, and meshed between the fingers in the filter paper until granular. The extraction was then continued, using the same solvent, for an

additional two hours.

At the end of the second extraction period the sample was removed and retained for the phytin phosphorus determination.

Most of the solvent from the boiling flask was removed by distillation and the residue poured into a small porcelain evaporating dish, 60 ml. capacity. The boiling flask was rinsed with two 10 ml. portions of 95 percent ethyl alcohol, heating to boiling each time, and adding the washings to the evaporating dish. This was repeated, using two 10 ml. portions of 75 percent ethyl alcohol.

After removing most of the solvent in the evaporating dish on a steam bath, three ml. of magnesium nitrate solution was added. The sample was heated over the steam bath until the residue was dry and then over a bunsen burner until well charred. The charred residue was ashed in a muffle furnace at 400° C. until most of the carbon was burnt off (about two hours), and then the temperature was raised to 600° C. and the sample heated until a white ash was obtained (about two hours).

The cooled ash was dissolved in two ml. of 10 N sulfuric acid, warming if necessary, and transferred quantitatively to a 100 ml. volumetric flask with the aid of distilled water. The digest was made up to volume with distilled water and mixed well. A 10 ml. aliquot of this digest was pipeted into a 125 ml. separatory funnel and the phosphorus determined colorimetrically as outlined in the isobutyl alcohol method.

Phytin Phosphorus. The extracted flour sample from the phosphatide extraction was air dried for 24 hours and transferred quantitatively to a 125 ml. glass stoppered erlenmeyer flask. Exactly 100 ml. of two percent hydrochloric acid solution was added to the sample and extracted on a mechanical shaker for two hours. The sample was centrifuged and filtered in the same manner as described under the Acid-Soluble Determination, discarding the first

portion of the filtrate.

The extracted gluten samples from the phosphatide extraction were air dried for 24 hours and transferred quantitatively to a micro Waring Blender bowl and 50 ml. of two percent hydrochloric acid solution added. The samples were extracted in the Waring Blender for 15 minutes and then transferred quantitatively to 125 ml. glass stoppered erlenmeyer flask, using 50 ml. of two percent hydrochloric acid to effect the transfer. The samples were extracted for an additional two hours on a mechanical shaker, centrifuged, and filtered in the same manner as the flour samples. The procedure for both the flour and gluten samples were identical after this stage.

A 20 ml. portion of the filtered extract was pipeted into a 50 ml. graduated conical tipped centrifuge tube. One drop of phenopthalein indicator, one percent solution in 95 percent ethyl alcohol, and two ml. of 5 N sodium hydroxide was added. The solution was adjusted with 1 N sodium hydroxide and 1 N hydrochloric acid until one drop of acid made the solution colorless and then diluted to 25 ml. volume with distilled water. Five ml. of the ferric chloride reagent was added, swirling the tube during the addition. A small stirring rod was introduced and the tubes placed in a boiling water bath for 15 minutes, stirring occasionally to promote flocculation of the ferric phytate. The tubes were cooled in a cold water bath, the stirring rods washed with two ml. of 0.6 percent hydrochloric acid solution, and the tubes centrifuged in a small laboratory centrifuge (minimum 1800 r.p.m.) for 20 minutes. The clear supernatant was poured off and the ferric phytate precipitate washed with five ml. of 0.6 percent hydrochloric acid delivered from a pipet so as to disperse the precipitate. The walls of the tube were washed with an additional two ml. of the 0.6 percent hydrochloric acid. The tubes were centrifuged a second time for 20 minutes as described above and the clear supernatant discarded.

The precipitate was suspended in five ml. of hot distilled water. Two ml. of 1 N sodium hydroxide was added and the tubes placed in a boiling water bath for 15 minutes with occasional stirring. The hot solution was filtered through seven cm. filter paper, Whatman No. 42, collecting the filtrate in a 100 ml. Kjeldahl flask. The centrifuge tube was washed with three five ml. portions of hot distilled water, decanting through the filter each time. The filter paper was washed with three additional five ml. portions of hot distilled water. The sample was digested and phosphorus determined colorimetrically as outlined under the Reduced Molybdate Method.

Nucleic Acid Phosphorus. Acid-soluble phosphorus determination includes phytin, inorganic, and ester type phosphorus. Therefore, the value for acid-soluble phosphorus plus the value for phosphatide phosphorus subtracted from the value for total phosphorus was made in order to estimate the amount of nucleic acid phosphorus.

Ester Phosphorus. The sum of the values for phytin and inorganic phosphorus was subtracted from the value for acid-soluble phosphorus in order to estimate the phosphorus present in the sample in the form of carbohydrate esters of phosphoric acid.

## Lipid Extract (19)

Procedure. A three g. sample of flour was weighed into a 125 ml. glass stoppered erlenmeyer flask and 30 ml. of water-saturated n-butyl alcohol added. This was shaken on a mechanical shaker for 45 minutes. The extract was centrifuged in 50 ml. conical centrifuge tubes for five minutes in a small laboratory centrifuge at low speed. A 20 ml. aliquot of the clear supernatant was pipeted into 125 ml. glass stoppered erlenmeyer flask and four, six mm., glass beads introduced. The solvent was evaporated to near dryness over a steam

cone using a vacuum to hasten the process. The residue was dissolved in five ml. of chloroform and filtered through a sintered glass filter of medium porosity with the aid of filter aid into a tarred 50 ml. glass beaker. The flask was rinsed five times with two ml. portions of chloroform and added to the filter. The filtrate was evaporated to near dryness over a steam cone using a small stream of steam. The residue was dried in a vacuum oven at 50° C. for one hour. The residue was weighed and percent lipid extract reported on a 14 percent moisture basis.

Results and Discussion. Data obtained for the percent lipid extract of the flour samples obtained by the method outlined above is submitted in Table 12.

The amounts of lipids extracted from the flours agree reasonably well with those reported by Meeham and Mohammad (19). The range in the flour samples in this study was 1.33 percent to 2.12 percent with an average percent lipids extract of 1.38 percent. The coefficient of correlation between adjusted loaf volume and percent lipid extract was insignificant ($r = 0.104$ ns). The conclusion was made that by the method of extraction employed in this study, the total percent lipid extract had little affect on flour quality for these samples.

Due to the low correlation that existed between percent lipid extract and adjusted loaf volume, no more work was pursued along this line. However, it is suggested that further work could be conducted to find new and better methods of lipid extraction than the methods now available. Also, further work should be conducted in fractionation of the extractable lipids and identification of these fractions and a study made of their relationship to flour quality.

Table 12. Summary of lipid data extracted by water-saturated n-butyl alcohol.

| Station | Variety | | | | | |
|---|---|---|---|---|---|---|
| | Bison | Pawnee | Ponca | Concho | RedChief | Average |
| | Lipid Extract (percent)[1] | | | | | |
| Manhattan | 1.286 | 1.273 | 1.242 | 1.248 | 1.164 | 1.243 |
| Hays | 1.342 | 1.487 | 1.347 | 1.405 | 1.313 | 1.379 |
| Colby | 1.535 | 1.424 | 1.409 | 1.396 | 1.299 | 1.413 |
| Garden City | 1.337 | 1.731 | 1.250 | 1.320 | 1.224 | 1.372 |
| Mound Valley | 1.300 | 1.355 | 1.456 | 1.349 | 1.050 | 1.302 |
| Thayer | 1.342 | 1.641 | 1.448 | 1.472 | 1.796 | 1.540 |
| Belleville | 1.757 | 1.539 | 1.437 | 1.201 | 1.145 | 1.416 |
| Canton | 1.229 | 1.400 | 1.389 | 1.212 | 1.487 | 1.343 |
| Average | 1.391 | 1.481 | 1.372 | 1.325 | 1.310 | 1.376 |

1. 14 percent moisture basis.

# THE RELATIONSHIP OF TYPES OF PHOSPHORUS IN WHEAT FLOURS TO FLOUR QUALITY

by

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The concentration of different types of phosphorus in the flour and gluten fraction of forty hard red winter wheats was determined, and the relationships of these types of phosphorus to the quality of the flour samples for bread-baking purposes were investigated. The types of phosphorus included were total, acid-soluble, nucleic acid, phytin, inorganic, phosphatide, and ester. Many of the flour samples were of poor baking quality as shown by the coefficient of correlation ($r = 0.54$) between loaf volume and percent flour protein. Total, acid-soluble, and phytin phosphorus determinations were made by a reduced molybdate colorimetric procedure. Acid-soluble phosphorus includes inorganic, phytin, and ester phosphorus. Inorganic and phosphatide phosphorus determinations utilized a colorimetric method involving extraction of a molybdenum blue complex with isobutyl alcohol. Nucleic acid and ester phosphorus were obtained by differences of other types of phosphorus.

An adjusted loaf volume that eliminated a variable, protein, was used as a single numerical evaluation of the quality of the flour samples.

The average distribution of the types of phosphorus in the flour, based on the total phosphorus in the flour and expressed as mg. per g. of flour, was acid-soluble 38.3 percent, nucleic acid 57.4 percent, phytin 4.9 percent, inorganic 3.0 percent, phosphatide 4.3 percent, and ester 30.4 percent. The average distribution of the types of phosphorus in the gluten, based on the total phosphorus in the gluten and expressed as mg. per g. of gluten, was acid-soluble 32.4 percent, nucleic acid 59.8 percent, inorganic 14.5 percent, phosphatide 7.9 percent, and ester 17.9 percent. No phytin phosphorus could be detected in the gluten by the method employed in this study.

Location and variety generally both were significant factors in determining the concentration of the different types of phosphorus in the flour and gluten.

Location generally was more important than variety in determining the concentration of the different types of phosphorus.

The simple coefficients of correlation between concentration of types of phosphorus in the flour and adjusted loaf volume indicated that total and nucleic acid phosphorus were the only types of phosphorus that appreciably affected flour quality. The phosphorus correlation coefficients between adjusted loaf volume and total and nucleic acid were -0.64 and -0.51, respectively. The concentration of total phosphorus in the flour was a more reliable measure of flour quality than flour protein content. Acid-soluble and inorganic phosphorus in the flour, though significantly correlated with adjusted loaf volume, were of too low a magnitude for any practical purpose.

The simple correlations between all types of phosphorus, with the exception of nucleic acid phosphorus, in the gluten and adjusted loaf volume were highly significant. Exceedingly high correlations existed between adjusted loaf volume and acid-soluble ($r = -0.73^{***}$), inorganic ($r = -0.71^{***}$), ester ($r = -0.69^{***}$), and phosphatide ($r = 0.56^{***}$) phosphorus in each case. Therefore, multiple and partial correlations were performed in order to determine which of these types of phosphorus were contributing most to the baking quality of the flour. However, since inorganic and ester phosphorus make up the total amount of acid-soluble phosphorus in the gluten, acid-soluble phosphorus was eliminated from the multiple linear regression.

The multiple correlations showed little increase over the simple correlation between adjusted loaf volume and inorganic phosphorus. The multiple correlation coefficients between adjusted loaf volume and the variables, inorganic and phosphatide phosphorus and between adjusted loaf volume and inorganic, phosphatide, and ester phosphorus as variables were $0.76^{***}$ and $0.77^{***}$, respectively.

Partial correlations showed that adjusted loaf volume was significantly correlated (-0.37*) with inorganic phosphorus when phosphatide and ester phosphorus were held constant. A similar positive partial correlation (0.33*) was obtained for the phosphatide phosphorus when inorganic and ester phosphorus were held constant. The insignificant partial correlation (-0.22 ns) obtained for ester phosphorus indicated that this type of phosphorus did not appreciably affect baking quality when inorganic and phosphatide phosphorus were held constant.

Both inorganic and phosphatide phosphorus contributed to the increase in the value of the multiple linear regression as indicated by the F value of 6.79* when phosphatide phosphorus was added to the effect of inorganic phosphorus. However, little was contributed to the multiple linear regression between adjusted loaf volume and the inorganic and phosphatide phosphorus by addition of the ester phosphorus. This was based on the F value (1.75 ns) obtained when ester phosphorus was included with the inorganic and phosphatide phosphorus in the multiple linear regression.

The relationships that existed between types of phosphorus and flour protein content generally approached similar relationships that existed between adjusted loaf volume and types of phosphorus, except that in most cases the former coefficients of correlations were of lower magnitude and of opposite sign.